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Antioxidant and Biological Activity of Duchesnea Indica (Andr.) Focke Extracts.

Ra JH[1], Nakamura M[1], and Kim JS[1, 2]*.

[1]Majors in Plant Resource and Environment, College of Applied Life Sciences, SARI, Jeju National University, Jeju 63243, Republic of Korea.
[2]The Research Institute for Subtropical Agriculture and Biotechnology, Jeju National University, Republic of Korea.

ABSTRACT

In this study, we used Duchesnea indica (Andr.) Focke (D. indica), which belongs to the Rosaceae family. To investigate the bioactivity of D. indica, we extracted by methanol and partitioned with n-hexane, ethyl acetate, n-butanol, and distilled water. DPPH scavenging activity, reducing power, total phenol and flavonoids contents, antimicrobial properties, α-glucosidase and α-amylase inhibitory activity were evaluated. The highest antioxidant content in the DPPH assay was the n-butanol fraction (RC_{50} = 1.67±0.4 µg/ml). Regarding phenol and flavonoid contents, the n-butanol fraction showed 4,274±21 mg GAE/g (total phenol content), while the ethyl acetate fraction showed 636±43 mg QE/g (total flavonoid content). In terms of α-glucosidase, α-amylase inhibition, and reducing power, the n-butanol fraction was found to have the highest inhibition and reducing capability. For the antimicrobial test, the ethyl acetate fraction demonstrated antimicrobial activities against Escherichia coli and Klebsiella pneumonia at minimum inhibitory concentration (MIC) value of 250 µg/ml. In addition, the n-butanol fraction inhibits Staphylococcuepidermidis effectively (MIC value of 8 µg/ml). If more experiments were conducted, D. indica could be proven a good candidate for its natural antioxidant and antimicrobial properties and ability to inhibit α-glucosidase and α-amylase, as opposed to a synthetic agent, such as BHT or acarbose.

Keywords: Antioxidant Activity, Antimicrobial Activity, Duchesneaindica (Andr.) Fock

Corresponding author

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INTRODUCTION

Recently, as people's attention to healthy diets has increased, the concept of "antioxidants" or the "antioxidative effect" has become widely known. In addition, reactive oxygen species (ROS), such as super oxide (·O^-) and peroxyl radical (ROO·), have become known to many people [1]. ROS occur naturally through normal metabolic function, or they are induced by an external stimulus that has an unpaired electron at the center [2], [3]. Free radicals are causative factors innumerous diseases, including cardiovascular disease, cancer, osteoporosis, degenerative disease, diabetes, and many others [4]. The human body defends itself by producing enzymes, such as superoxide dismutase, glutathione peroxide, and catalase, to arrest ROS, but when the capacity to control is overtaken, it contributes serious oxidative damage [3]. In addition, some reports show that related ROS damage is produced not only endogenously, but also by various food products or perished food caused by microorganisms, indicating clearly that the ROS could induce harmful effects on the body and lead to poor food quality [5]. As a result, synthetic antioxidative agents, such as butylated hydroxyl anisole (BHA) or butylated hydroxyl toluence (BHT) and calcium benzoate as antimicrobial agents, have been used as long-term safety panacea to combat serious diseases. However, these synthetic agents have been reported to have possible adverse effects [6]. Hence, the associated natural antioxidants play a major role in protecting from damage caused by oxidation or microbial spoilage. Many recent studies have suggested that edible and medical plants possess higher phenol or flavonoid contents [7], and they discovered how flavonoid interacts with oxidative agents [8]. Especially, people have been recognizing the potency of herbal plants and developing efficacious medical treatments for a long time.

Duchesnea indica (Andr.) Focke (D.indica) belongs to the Rosaceae family, and it is one of two species in the genus with Duchesneschrysantha, called "mock fruits" or "false strawberry" because its berry is tasteless but the shape is similar to the strawberry. D.indica is an indigenous herbal plant in south central Asia, but it is widely distributed around the world [9]. In China and Japan, D. indica has been mostly used to treat tumors as folkloric medicine [10], to cure dentalgia and congenital fever, and to stanch blood in Korea [11]. In addition, the wide variety of properties of D. indica has been revealed. A recent report suggested that the methanol extracted from D. indica was able to preserve highly the CCD-986Sk cell, referred to as the human skin fibroblast cell, from H_2O_2-induced skin damage, indicating its use in protecting from oxidation-enhanced dermal damage [12]. In supplement, Yang et al. (2010) performed the anti-wrinkle and whitening effect using the ethyl acetate and n-hexane fractions, showing much higher collagenase inhibitory activity for the anti-wrinkle effect in 10 mg/ml compared to retinyl acetate used as a positive control (83.2±5.34 for the ethyl acetate fraction and 42±2.98 for retinyl acetate). In addition, there was slightly lower tyrosinase inhibitory activity for a whitening effect compared to L-ascorbic acid (30.6±2.63 for the ethyl acetate fraction and 36.6±3.01 for L-ascorbic acid) [11]. Through their study, D. indicais expected to be a possible and useful anti-aging and cosmeceutical agent. D. indicais now utilized effectively not only in the pharmaceutical but also many other fields. However, although there are some reports about D. indica, to the best of our knowledge, there is no literature on its antioxidant properties, α-glucosidase inhibitory activity, and antimicrobial characteristics with four fractions (n-hexane, ethyl acetate, n-butanol, and distilled water) and an extract (methanol). We performed a number of in vitro assays to evaluate DPPH radical scavenging activity, reducing power, total phenol and flavonoid contents, α-glucosidase and α-amylase inhibitory activity, two-fold dilution, and the disk diffusion method.

MATERIALS AND METHODS

Chemicals

Gallic acid, 4-nitrophenyl-a-$_D$-glucopyranoside (pNPG), α-glucosidase, and quercetin were obtained from Sigma Chemical Co., Ltd. (St. Louis, MO). In addition, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), BHT, potassium ferricyanide, ferric chloride, and trichloroacetic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals and reagents used in this study were analytical grade or better.

Plant material

Whole D. indica plants were kindly contributed as Jeju habitual biological resources used as cosmetic raw materials from Tamnamo Co., Ltd. (Jeju, Korea) in 2013. The sample was dried at 45 ºC for 4 days and

finely grinded by blender. Coarsely pulverized plant powder was extracted by a volume of methanol 10 times greater than the sample weight by ultrasonic bath (Power sonic 520, Hwashin Co., Ltd., Korea), and this was repeated three times (1 h each). After that, the extraction was concentrated to dryness using a rotary vacuum evaporator (hHei-VAP Precision 280rpm, Heldolph, Germany). The crude extract was suspended by distilled water, then partitioned into n-hexane, ethyl acetate, n-butanol (water-saturated BuOH), and distilled water. The extract and partitioned fractions were stored at -20 ºC until further analysis. A voucher specimen (No. Ra-09-01) of the plant was deposited at the Plant Resource and Environment, Jeju National University.

DPPH radical scavenging capacity

This method was measured using the technique of Ra et al. (2017) [13]. The volume was increased up to 4 ml by adding plant extract or fractions to methanol. Prior to incubating for 30 min, 1 ml of 0.15 mM DPPH was added. Finally, the absorbance was measured at 517 nm by UV spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The required amount of antioxidants to reduce by 50% of the DPPH radical concentration was expressed by RC_{50}, and the percent of DPPH radical scavenging activity of the samples was calculated according to the formula:

$$\text{Scavenging activity (\%)} = [(A_c - A_s)/A_c] \times 100$$

where $A_s$is the absorbance value of the tested sample and $A_c$is the absorbance value of the blank sample, respectively. Percent inhibition after 30 min was determined according to the concentration. A lower RC_{50} value indicates greater antioxidant activity.

Evaluation for reducing power

Reducing power was analyzed by the method of Nakamura et al. (2017) [14]. An appropriate dilution of methanol extract and each fraction were added in a test tube that contained 500 µl of 0.2 M sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide. Incubation at 50 ºC followed for 20 min, and the resultant was mixed with 2.5 ml of 10% trichloroacetic acid. Five hundred of the supernatant, distilled water, and 1% ferric chloride (100 µl) were mixed. After that, the absorbance of reducing power was measured at 700 nm by UV spectrophotometer, where BHT was used as a positive control.

Determination of total phenol content

Total phenol content was determined according to the Folin-Ciocalteu method of Nakamura et al. (2018) [15]. First, 50 µl of the Folin-Ciocalteu reagent was mixed with each sample solution, and they were stored at room temperature for 5 min. After 5 min, 300 µl of 20% sodium carbonate was added, and the mixtures were incubated again for 15 min. The mixture was vigorously mixed with a vortex, and absorbance was subsequently measured using a UV spectrophotometer at 725 nm. Total phenol content was calculated using a gallic acid equivalent (mg GAE/g), and the standard curve was obtained from 1 g of extract per gallic acid.

Determination of total flavonoid content

Total flavonoid content was assessed using the method of Nakamura et al. (2018) [15]. One hundredµl of 10% aluminum nitrate and 1 M of potassium acetate were put into a test tube and shaken vigorously. Next, 4.6 ml of 80% ethanol was spiked and stored at an ambient temperature for 40 min. The absorbance rate was a wavelength of 415 nm, as determined by UV spectrophotometer. The total flavonoid content was expressed by quercetin equivalents (mg QE/g).

α-Glucosidase inhibitory activity

The inhibitory activity of α-glucosidase was determined according to the method of Nakamura et al. (2016) [16]. Each 50 µl of 0.2 M potassium phosphate buffer (pH 6.8) and 0.2 U/ml α-glucosidase enzyme solution was added to the extract and each fraction. After 15 min of incubation at 37 ºC, 0.1 ml of 3 mM p-nitrophenyl α-D-glucopyranoside (pNPG) was mixed in. After stopping the reaction by adding 0.1 M sodium carbonate (750 µl), the absorbance was determined at 405 nm. The negative control, which has no sample

solution; blank, which has no substrate; and acarbose were used as positive controls. The inhibitory activity of α-glucosidase was determined by IC_{50} (inhibition concentration), and the inhibition rate was calculated below.

$$\text{Inhibition ratio (\%)} = [1 - (A_s/A_b)/A_c] \times 100$$

Where A_s represents the absorbance of the sample that was decanted in this study, A_b is the absorbance of the blank without pNPG, and A_c is the control value without a sample solution. Also, acarbose was used as comparing propose.

α-Amylase inhibitory activity

The α-amylase inhibitory activity assay was adopted according to Kim et al. (2011) [17] and performed on a petridish composed of 1% (w/v) starch dispersed in 1.5% agar. Sterile Whatman No. 1 (8 mm) disc papers were individually placed on agar plates, and then 10 µl of the samples was applied to the filter paper disc. After incubation at 37 ºC for 72 h, starch plates were stained by flooding with iodine solution (5 mM I_2 in 3% KI) for 15 min at room temperature. Iodine was removed from the plates by washing with distilled water. Amylase activity was determined by observing the zone diameter of the hydrolyzed areas around the wells. As comparison, acarbose was used as positive control.

Antimicrobial testing

The bacterial strains used in this experiment were obtained from Korean Agricultural Culture Collection (KACC, Suwon, Korea). The experiment was set by adopting the method of Ra et al. (2018) [18]. The obtained strains include three gram-negative bacteria and four gram-positive bacteria, as follows: Escherichia coli (KACC 14818), Klebsiella pneumonia (KACC 14816), and Enterobacter cloacae (KACC 11958) for gram-negative bacteria and Kocuria rhizophila (KACC 14744), Bacillus subtilis subsp. Spizizenii (KACC 14741), Micrococcus luteus (KACC 14819), and Staphylococcus epidermidis (KACC 14822) for gram-positive bacteria. The minimum inhibitory concentration (MIC) value was determined using the serial two-fold dilution method, expressing the minimum inhibitory concentration.

The disc diffusion assay was applied using the method of Ćavaret al. (2012) [19] by using the n-butanol fraction against Staphylococcus epidermidis. The study was carried out by spreading out 50 µl of pre-incubated Staphylococcus epidermidis to the cultural medium. The n-butanol fraction and tetracyclin were used as positive controls and brought up to 60 µg/ml per disk (6 mm in diameter). After drying spontaneously, the disk was put on the cultural medium, where it remained at 30 °C. The clear zone (mm) produced around the disk was observed in 30 h.

Statistical analysis

All data in this study are expressed as the mean ± standard deviation of representative duplicate experiments, and statistical significance was determined by one-way analysis of variance, with $P < 0.05$ considered significant. When $P < 0.05$, Duncan's multiple range test was performed to determine the significance between mean values. (All analyses were performed using the SAS Statistical Package for the Social Sciences, Ver. 20.0 [SAS institute Inc., Cary, NC, USA]).

RESULTS AND DISCUSSIONS

The analysis of antioxidant activity

The required amount to inhibit 50% of DPPH radical activity was expressed as RC_{50} and depicted in Table 1. The smaller the RC_{50} value, the higher the DPPH radical scavenging activity. DPPH radical scavenging activity was higher from n-butanol and ethyl acetate fractions, methanol extract, n-hexane, and aqueous fraction.As shown in Table 1, although each extract and fraction of D. indica was not higher than α-tocopherol (1.41±0.03 µg/ml), a reference substance, all showed remarkable antioxidant activity. Specifically, the n-butanol fraction demonstrated a slightly lower value (1.68±0.4 µg/ml) than α-tocopherol. In addition, n-hexane exhibited the lowest DPPH radical scavenging activity. The fact may elucidate that active components against oxidation would be polar compounds. Further, the RC_{50} value is increased as phenol content becomes higher.

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Some authors described that total phenol content and DPPH radical scavenging activity lack a correlation [20], [21], but our result was in good agreement with other literature [22], [23]. It might be high DPPH radical scavenging activity was possibly consistent with total phenol content in our study. In our experiment, as expected, n-butanol fraction, which has the highest phenol content, exhibited the highest reducing power (Fig. 1). Meanwhile, methanol extract and the ethyl acetate fraction showed the second- and third-highest reducing ability, respectively. The reducing power was higher in a dose-dependent manner, and methanol extract and the ethyl acetate fraction demonstrated similar or slightly lower values than BHT when used as a positive control. In addition, n-butanol fraction showed a higher value than BHT.

Table 1: The result of total phenol and flavonoid, α-glucosidase inhibitory activity and DPPH radical scavenging activity

Extract and Fractions	TPC (mg GAE/g)*	TFC (mg QE/g) **	IC_{50} (µg/ml)***	RC_{50} (µg/ml)****
Methanol extract	2108±75^{b*****}	246±1^{c}	0.29±0^{a}	3.28±0.25^{ab}
n-Hexane fraction	611±43^{e}	370±11^{b}	>100^{c}	38.69±0.24^{d}
Ethyl acetate fraction	1782±86^{c}	636±5^{a}	0.73±0.03^{a}	5.22±0.31^{b}
n-Butanol fraction	4274±21^{a}	96±5^{d}	0.14±0.01^{a}	1.68±0.40^{a}
Aqueous fraction	1138±70^{d}	73±5^{d}	0.73±0.04^{a}	11.58±0.43^{c}
BHT				43.66±1.45^{e}
Tocophenol				2.98±0.02^{ab}
L-Ascobic acid				1.21±0.01^{a}
Acarbose			96.32±1.21^{b}	

* TPC: Total phenolic content analysed as gallic acid equivalent (GAE) mg/g of extract. ** TFC: Total flavonoid contents analysed as quercetion equivalent mg/g of extract. *** IC_{50}: Amount required for 50% reduction of α-glucosidase. **** RC_{50}: Amount required for a 50% reduction of DPPH free radicals after 30 min. ***** Values are the average of duplicates.

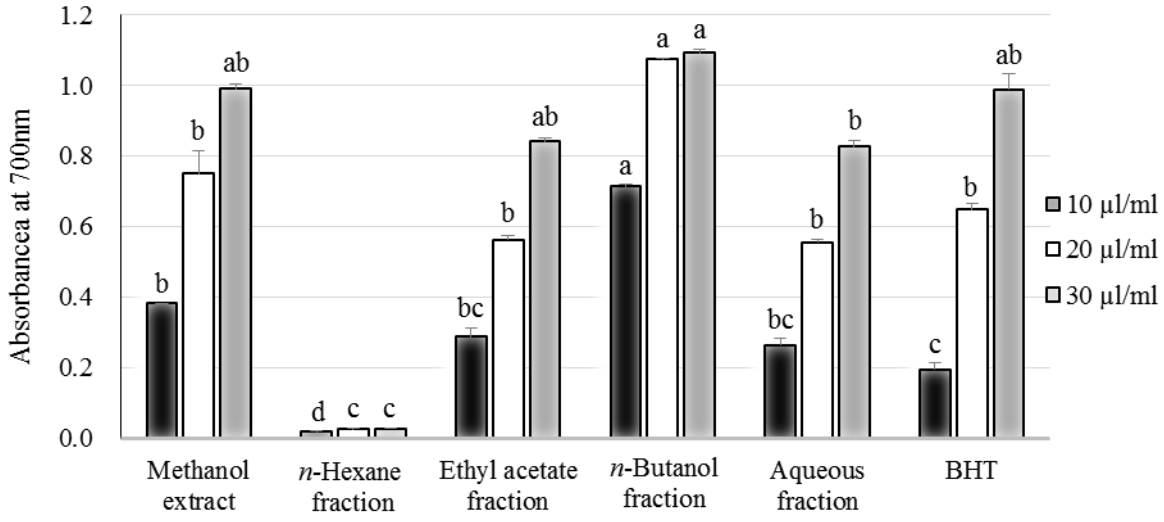

Fig 1: **Reducing ability of Duchesnea indica (Andr.) Focke depend on extract and fractions.**

The reducing power shows that there is a correlation between phenol contents [24]. Our result also showed the same trend. Reducing power increased proportional to the total phenol content. However, interestingly, while the ethyl acetate fraction exhibited much higher DPPH scavenging activity (5.34±0.29 µg/ml) and total phenol content (1,782±86 mg GAE/g) compared to the aqueous fraction (16.58±0.43 µg/ml for DPPH scavenging activity and 1098±0 mg GAE/g for total phenol content), a prominent difference in the reducing power assay was not shown. Considering the ethyl acetate fraction has more propensity to attract active compounds against scavenging free radicals than the aqueous fraction, according to previous reports

[17], the aqueous fraction might contain more compounds that effectively work better as reductants than the ethyl acetate fraction in our study.

Total phenol and flavonoid contents

In terms of total phenol contents, the n-butanol fraction exhibited the highest contents (4,274±21 mg GAE/g), followed by the ethyl acetate (1,782±86 mg GAE/g), aqueous (1,098 mg GAE/g), and n-hexane (611±43 mg GAE/g) fractions. The aqueous and n-hexane fractions revealed slightly lower values than the other fractions. In terms of total flavonoid contents, contrary to phenol contents, the ethyl acetate fraction showed the highest flavonoid contents (655±43 mg QE/g). In addition, even if the n-butanol fraction exhibited the highest phenol contents, the total flavonoid contents were lower than the n-hexane fraction. From Table 1, the highest total phenol contents were seen in the n-butanol fractions. However, Hyun et al. (2015) demonstrated total phenol content using crowberry (EmpetrumnigrumL.) leaves and stems with an extract (methanol) and four fractions (n-hexane, ethyl acetate, n-butanol, and aqueous fractions), where methanol extract showed the highest total phenol content (1,170±4 mg GAE/g) [25]. Although our result showed that the n-butanol fraction exhibited the highest content, their result demonstrates a different outcome. Furthermore, our result demonstrated a much higher total phenol content than did theirs (4,274±21 mg GAE/g for D. indica and 1,170±4 mg GAE/g for Empetrumnigrumin methanol extract, 611±43 GAE/g for D.indica and 111±10 GAE/g for Empetrumnigrum in the n-hexane fraction, 1,782±86 GAE/g for D.indica and 817±25 GAE/g for Empetrumnigrumin the ethyl acetate fraction, 4,274±21 GAE/g for D.indica and 950± 76 GAE/g for Empetrumnigrumin the n-butanol fraction, and 1,138±70 GAE/g for D.indica and 160±8 GAE/g for Empetrumnigrumin distilled water). It clearly elucidated, as Phanget al. (2013) described, that the solvents, the kinds of plants, and the plant parts significantly affect total phenol content andthey can be extracted; hence, the n-butanol fraction is the ideal solvent for D. indica [26].Moreover, compared to the research on the same Rosacea family by Froehlicher et al. (2009), who investigated the antioxidant properties of the fresh fruit, dry fruit, flower top, and flowering bud of Crataegusmonogyna fractionated into ethyl acetate, our result (1,782±86 mg GAE/ g in ethyl acetate fraction) showed a much higher total phenol content compared to each part of Crataegusmonogyna (6.089±32.6 mg GAE/g for fresh fruit, 3.603±8.7 mg GAE/g for dry fruit, 20.772±29.8 mg GAE/g for the flowering top, and 15.630± 61.9 mg GAE/g for the flower bud) [27].

Table2: **The Minimum Inhibitory Concentration (MIC) of each extract and fractions for Duchesnea indica (Andr.) Focke against tested strain.**

	MIC (µl/ml)						
	-			+			
	E.c*	K.p*	E.cl*	K.r*	B.s*	Mi*	S.e*
Methanol extract	500	>1000	500	250	250	1000	31
n-Hexane fraction	>1000	>1000	>1000	>1000	>1000	>1000	>1000
Ethyl acetate fraction	250	1000	250	125	250	125	31
n-Butanol fraction	63	500	125	125	125	125	8
Aqueous fraction	>1000	>1000	500	1000	>1000	1000	63
Tetracycline	8	8	8	8	8	8	8

The MIC values against bacteria were determined by the serial two-fold dilution method. The growth of the bacteria were evaluated by the degree of turbidity of the culture with the naked eye.

*E.c: Escherichia coli (KACC 14818), K.p:Klebsiella pneumoniae (KACC 14816), E.cl: Enterobacter cloacae (KACC 11958), K.r:Kocuria rhizophila (KACC 14744), B.s: Bacillus subtilis subsp. spizizenii (KACC 14741), Mi: Micrococcus luteus (KACC 14819), S.e: Staphylococcus epidermidis (KACC 14822).

The small discordance between n-hexane in total flavonoid content is led by a non-exact specificity to detect the flavonoid content. Işık et al. (2013) investigated and compared the immobilized horseradish peroxidase (HRP) method and Folin-Ciocalteu method in terms of total phenol content and DPPH assay and anticipated non-phenolic compounds, such as citric acid, ascorbic acid, and sulfite, interfering with phenolic compounds, resulting in a higher absorbance of the phenol contents [28]. Likewise, there would be a possibility that these substances would compete with the flavonoid content in our assay. From Table 1, the ethyl acetate fraction showed the highest amount of flavonoid content (636±5 mg QE/g), whereas the n-butanol fraction demonstrated the highest total phenol content (4,274±21 mg GAE/g) and showed a lower

flavonoid content (96±5 QE/g). In addition, the n-butanol fraction exhibited the highest DPPH radical scavenging activity and α-glucosidase inhibitory activity. It might be inferred that some of the active compounds that could not be detected by total flavonoid assay work as significant radical scavenging and α-glucosidase inhibitory agents in our study.

α-Glucosidase and α-amylase inhibitory activity

The efficacy of α-glucosidase inhibitory activity is tabulated in Table 1. Most fractions and the extract exhibited significant α-glucosidase inhibitory activity, but the n-hexane fraction showed low inhibitory activity (>100µg/ml). In all fractions and the extract, the n-butanol fraction inhibited α-glucosidase at the highest amount (0.14±0.01 µg/ml). Rani et al. (2014) performedanα-glucosidase inhibitory assay with strawberry fruits (Fragariaananassa), and they divided them into three samples depending on the maturity stage (pre-ripening stage I [0.329±36.26 mg/µl], stageII [0.271±42.49 mg/µl], and stage III [0.154±33.12 mg/µl]) [29].

Compared to the n-butanol fraction in our study, the n-butanol fraction demonstrated greater α-glucosidase inhibition (0.14±0.01mg/µl) than all three stages in their investigation. In addition, methanol extract (0.29±0 mg/µl) in our observation exhibited a similar value with a pre-ripening stage II strawberry. This strong α-glucosidase inhibitory activity might be supported by inherent carbohydrate [30] or glycoside content, which hasa similar structure to carbohydrate and which acts as a substrate of α-glucosidase [31]. In addition, Hyun et al. (2014) investigated 40 plant-derived phenol and flavonoid contents to screen potential α-glucosidase inhibitors with structure-based molecular docking and found that quercetin had the most latent α-glucosidase inhibitory ability, followed by myricetin, and rutin (IC_{50} of 1.0±0.1 µg/ml, 3.2±0.1 µg/ml, 84.1±4.1 µg/ml, respectively) [32]. According to Liu et al. (2012), D. chrysantha, which is a closely related species sharing the same genus as D. indica, contains quercetin-3-O-β-glucoside [33], and Ohara et al. (2011) isolated active compounds from strawberry leaves (Fragariaananassa), the same family as D. indica, which contained quercetin-3-O-β-disaccharide and quercetin-3-O-β-glucopyranoside, derivatives of quercetin [34]. These research studies imply that D. indica may contain quercetin and contribute remarkably to α-glucosidase inhibitory activity in our study.

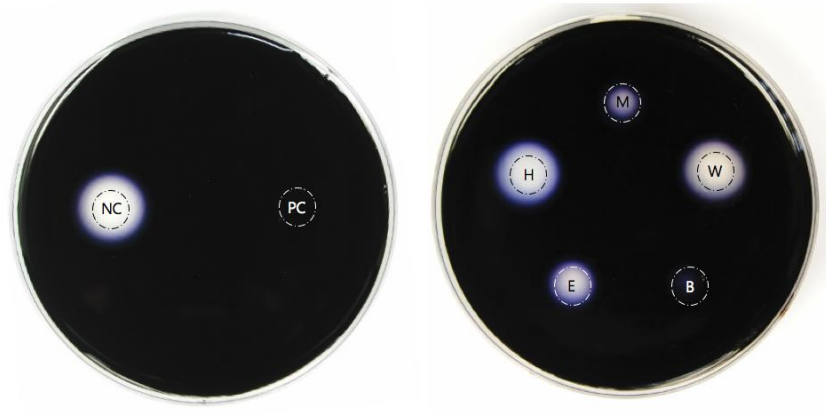

Fig 2: The inhibition of α-amylase for each extract and fractions.

NC:Negative Control, PC: Acarbose, M: Methanol extract, H: n-Hexane fraction, E: Ethyl acetate fraction, B: n-Butanol fraction, A: Aqueous fraction

As shown in Fig. 2, the n-butanol fraction exhibited the strongestα-amylase inhibitory activity, followed by methanol and the ethyl acetate, aqueous, and n-hexane fractions. Our result was the same as that of Wang et al. (2010), who demonstrated the α-amylase inhibitory activity of guava leaves and showed that the n-butanol fraction was the highest α-amylase inhibitory agent [35]. Kim et al. (2011) also investigated the α-amylase inhibitory ability of sorghum, foxtail millet, and proso millet [36], and their result suggested that α-amylase inhibitory activity correlates with total phenol content. From Table 1, the highest total phenol content was the n-butanol fraction, with the order being the same as that for α-amylase inhibitory activity. Considering this, it

can be predicted that there is also some correlation between total phenol content and α-amylase inhibition in the present study.

Antimicrobial testing

Recent research has proven that phenol also works as an antibacterial agent [37]. Hence, we additionally measured antibacterial properties. The gram-negative and -positive bacteria were chosen to measure the inhibitory abilities of D. indica with an extract and fractions. Although the n-hexane fraction did not show any antimicrobial activity toward the tested strain, the n-butanol fraction inhibited Kocuriarhizophila with a concentration of MIC 125 µg/ml. In addition, ethyl acetate fraction effectively works against Escherichia coli and Klebsiellapneumoniae (MIC 250 µg/ml). Generally, the n-butanol and ethyl acetate fractions demonstrated higher antimicrobial activity than methanol and the aqueous fraction. Especially, the n-butanol fraction showed a similar inhibition with tetracyclin, the synthetic microbial agent, of Staphylococcus epidermidis. To analyze further, we conducted the disc diffusion method employing Staphylococcus epidermidis and tetracyclin, which was 156±5.6 mm, and the n-butanol fraction, which was 180.5±4.9 mm, exhibited greater inhibitory activity (Fig. 3).

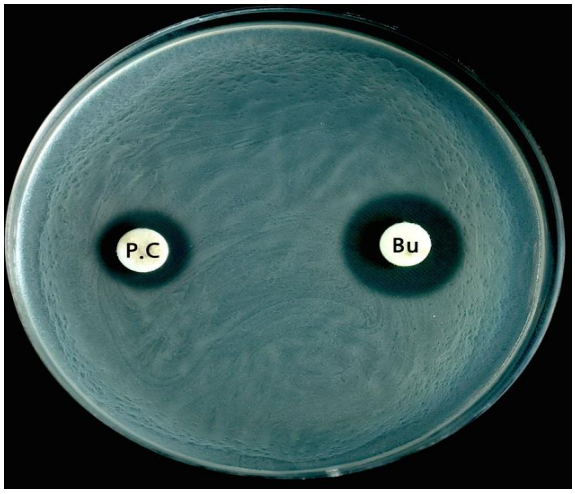

Fig 3: The inhibition effect of n-butanol fraction in disk diffusion assay against Staphylococcus epidermidis. (P.C : Tetracycline, Bu : n-Butanol fraction)

From the result, the extract and fractions of D. indica are more effective against gram-positive bacteria than gram-negative bacteria. Staphylococcus epidermidisis especially susceptible, and D. indica extract and fractions may include constituents that effectively inhibit Staphylococcus epidermidis growth. Although the ethyl acetate fraction demonstrated higher antimicrobial activity than the methanol extract considering total phenol contents, as tabulated in Table 1, the inhibition of bacterial multiplication mostly rose in proportion to increased total phenol content. This implied that there would be some matter of correlation between total phenol content and antimicrobial activity in our study. Through our observation, the ethyl acetate fraction might contain different substances rather than phenolic compounds, possibly showing relatively higher antimicrobial activity than methanol extract. The efflux pump, which is one of the causative factor microbes gaining multidrug resistance (MDR) and which pumps out extruded antimicrobial agents to the external medium [38], might be affected by so-called “efflux pump inhibitors (EPIs)” that may exist in the ethyl acetate fraction. Shiuet al. (2013) isolated a new antibacterial compound calledolympicin A from HypericumolympicumL.cf.uniflorum and found that it has ability to impede the NorA multidrug efflux pump system through a radio metric accumulation assay that measured the accumulation of ^{14}C-enoxacin. It might be inferred that the ethyl acetate fraction contains such a substance [39].

CONCLUSION

The present study has revealed that D. indica possesses a significant amount of effective biological compounds that are probably derived, for example, from flavonoid or phenol content. Especially, the n-butanol fraction exhibited greater activity in each assay. It could be pointed out that the n-butanol fraction

could extract these compounds efficiently. It can be stated that D. indica should be regarded as a potent antioxidant, anti-diabetic, and antimicrobial agent with remarkable high flavonoid and phenol content. However, further investigation will be warranted to elucidate and determine the active components of D. indica so that its mechanism of inhibitory and scavenging activity will be clearer. Furthermore, an advanced in vivo investigation will be also needed.

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